GNR That Cause Serious Disease

Introduction

These Gram-negative rods we are about to discuss cause serious disease. The two most important organisms are *Escherichia coli* (which will be referred to only as *E. coli*) and *Pseudomonas aeruginosa* (which will be referred to only as *Pseudomonas*). We spend the most time on those organisms and the diseases they cause. They are common causes of infection, sepsis, and hospitalization. The other three organisms—*Klebsiella*, *Proteus*, and *Legionella*—get their own shorter discussions. Be careful: the similarities between organisms is deceptive and can confuse novice learners. The fact that they cause UTIs and pneumonia is not thematic, but rather coincidental. These bacteria are taught together because they cause serious medical disease, are common infections treated on clinical rotations, and have antigenic LPS. Learn each bacterium as a discrete organism causing discrete disease.

UTI	UTI AND STONES	PNEUMONIA	SKIN AND SOFT TISSUE	MENINGITIS
Pseudomonas	–	*Pseudomonas*	*Pseudomonas*	–
E. coli	–	–	–	*E. coli*
Klebsiella	*Klebsiella*	*Klebsiella*	*Klebsiella*	–
Proteus	*Proteus*	–	–	–
–	–	*Legionella*	–	–

Table 8.1: Gram-Negative Bacteria Organized by the Diseases They Cause

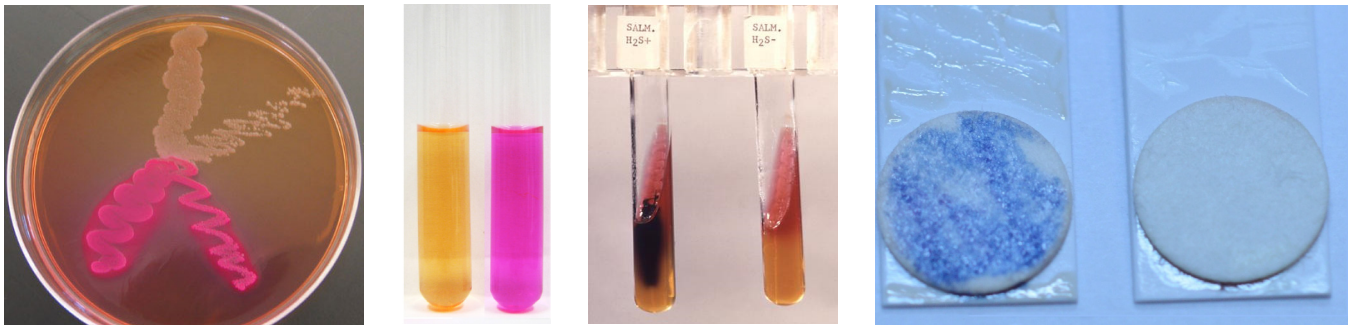
We start off with a more detailed version of laboratory diagnostics pertaining specifically to Gram-negative bacteria, then engage each bacterium one at a time.

Laboratory Microbiology

We started the discussion in Bacteria #4: *Laboratory Diagnosis*, but now want to round out the discussion of the tests that are performed on Gram-negative organisms—lactose fermentation, oxidase test, and production of H_2S.

Lactose fermentation. One of the key differentiators for Gram-negative organisms is whether they **ferment lactose**. Most Gram negatives do not. The two that do ferment lactose are ***E. coli*** and ***Klebsiella*** (and *Serratia* and *Enterobacter*, but don't learn them). This is determined by a MacConkey agar, in which the fermentation of lactose changes the medium from yellow to pink. A **pink MacConkey agar** means **lactose fermenter**.

Oxidase test. Strains of bacteria are either oxidase positive (OX+) or oxidase negative (OX–). Oxidase is a mechanism by which the organism can generate energy by using oxygen and an electron transfer chain, a rudimentary version of our mitochondrial oxidative phosphorylation. It isn't a virulence factor, it doesn't facilitate survival within phagosomes, it is just a means of generating energy. The organisms that are OX+ are ***Pseudomonas*** (this lesson), ***Neisseria*** and ***Moraxella*** (lesson #13: *Gram-Positive Rods*), and the comma-shaped rods ***Vibrio*** and ***Campylobacter*** (lesson #9: *GNR That Cause Diarrhea*). The oxidase test takes advantage of the presence of the oxidase enzyme to turn an indicator blue. This is merely a laboratory technique. In this lesson, all organisms are oxidase negative, except for *Pseudomonas*. To use oxygen to generate energy, the bacterium must not be anaerobic, by definition.



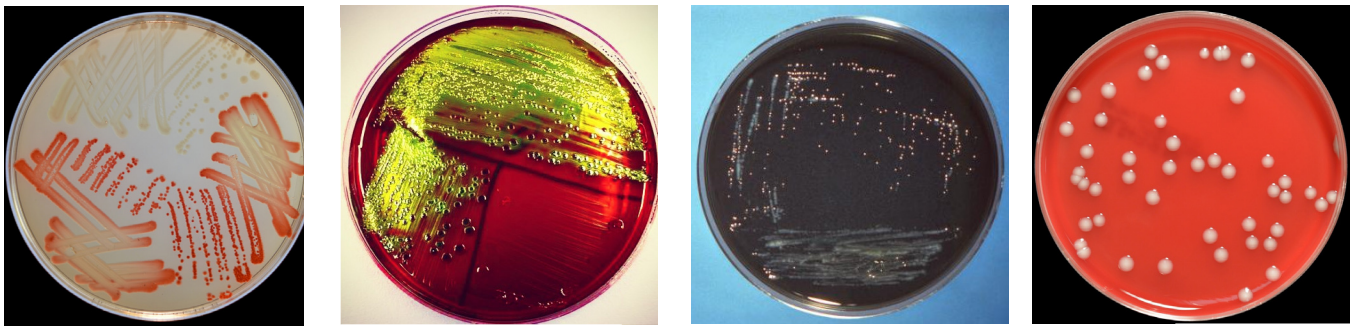
(a) (b) (c) (d)

Figure 8.1: Gram-Negative Laboratory Microbiology
This is the same text and images from lesson 4, repeated here for your reference. (a) MacConkey agar starts yellow. It turns red or pink in the presence of acid, indicating lactose fermentation. The colonies themselves will either be red (fermenter) or colorless (nonfermenter). (b) Urease test. If positive, the solution will turn from yellow to pink. (c) Tripe Sugar Iron tests for H_2S production. If positive, there will be black granules on the agar. (d) Oxidase test turns blue when the organism does produce oxidase. This may be performed on a tab, in a test tube, or by many other means.

H_2S production. Some organisms use something other than oxygen in their generation of energy. Organisms that use sulfur produce this gas, and so generate black precipitate on Triple Sugar Iron (TSI) agar. In this lesson, ***Proteus*** produces H_2S and none of the others does. The other bacterium that produces H_2S is salmonella (lesson #9: *GNR That Cause Diarrhea*). These bacteria are OX–, but being OX– does not mean the bacteria will produce H_2S (*Shigella* and *Yersinia*, for example, are oxidase negative and H_2S-production negative.)

	LACTOSE FERMENTERS	OXIDASE +	H_2S PRODUCTION
This lesson	*E. coli* *Klebsiella*	*Pseudomonas*	*Proteus*
Don't forget	~~*Serratia*~~ ~~*Enterobacter*~~	*Neisseria* and *Moraxella* *Vibrio* and *Campylobacter*	*Salmonella*
Results	MacConkey turns pink	Indicator turns blue	Black precipitate

Table 8.2: Gram-Negative Microbiology Laboratory Diagnosis
Strikethroughs are to remind you not to study or learn them.



(a) (b) (c) (d)

Figure 8.2: Agar Diagnosis
(a) *Serratia* colonies are red (the red colonies are on a red blood agar). (b) *E. coli* turns EMB agar green with a metallic sheen. (c) *Legionella* is white on BCYE, which is intensely black. BCYE is made for only *Legionella*. (d) *Klebsiella* will appear on a red blood agar as having a thick, mucoid appearance.

E. coli

E. coli is the most common and important of the genus. It is the quintessential poop bug that causes both poop disease and disease elsewhere. It has **intestinal manifestations** (watery, secretory diarrhea and bloody invasive diarrhea). It has extraintestinal manifestations, namely **urinary tract infections** and **neonatal meningitis**. We are going to discuss the details of *E. coli* microbiology in this lesson. In the next (Bacteria #9: *GNR That Cause Diarrhea*), we will use the information from this lesson and the lesson on toxins (Bacteria #3: *Toxins*) to contextualize *E. coli*'s role in diarrheal illness. Here we discuss the virulence and epidemiology.

Structure/Physiology. *E. coli* is a Gram-negative rod. It is one of the two **lactose fermenters**, and so turns a MacConkey agar from yellow to pink. Unique to *E. coli* is that it has a **green metallic sheen** on EMB agar. It happens to be catalase positive, so helps resist phagocytic killing (though because it is Gram-negative, catalase is not used to differentiate it from other bacteria).

Virulence. *E. coli* has a potent endotoxin and is why severe sepsis so often occurs in the setting of urinary tract infections. The *E. coli* that causes diarrhea produce exotoxins—EHEC makes Shiga toxin, ETEC makes heat stable and heat labile toxin (see #9: *GNR That Cause Diarrhea*). For the *E. coli* that cause extraintestinal manifestations, it is not the toxins, but additional virulence properties that enable them to cause disease outside of the GI tract. For those *E. coli* that cause **neonatal meningitis**, almost all cases demonstrate the **K1 capsular antigen.** For those *E. coli* that cause **urinary tract infections**, they all have some way of adhering to the epithelium, preventing elimination by the flow of urine. The **P-pilus** if *E. coli* has adhesin proteins that bind specifically to the urinary tract epithelium (P-pilus causes pee-pee disease).

Diseases. *E. coli* causes watery diarrhea, bloody diarrhea, urinary tract infections, and neonatal meningitis, and is one of the most common causes of septicemia. Being in the colon means that any perforation of the colon can lead to intra-abdominal abscesses, and translocation from the colon to the bloodstream can result in bacteremia.

Treatment. The treatment varies widely. For EHEC and ETEC, those of the gut, nothing need be done. For the urinary tract infections and meningitis, extended-spectrum penicillins (amoxicillin, ampicillin) and third-generation cephalosporins tend to work well. But there are extended-spectrum β-lactamase (ESBL)-resistant *E. coli* developing everywhere because the bug is so ubiquitous, and big-gun antibiotics may be required. Do not learn this antibiotic for that *E. coli*; learn this antibiotic for this diagnosis, then change the antibiotic based on sensitivities.

Klebsiella

Klebsiella has a large polysaccharide capsule which gives the colonies of *Klebsiella* growing on blood agar a **mucoid appearance.** *Klebsiella* is a **lactose fermenter**, so will turn pink on MacConkey agar. It is immotile and is **urease positive**. It is part of the normal colonic flora and causes UTIs. Be careful; this is NOT just another *E. coli.*

Like *E. coli* and *Proteus*, it is one of the most common causes of **urinary tract infections**. Like *Proteus,* it is urease positive, and because it is urease positive it can cause struvite stones. Like *E. coli*, it is found in the normal colonic flora, and so the gut bugs' exit (the anus) is near the urethral entrance. *Klebsiella* ascends the urethra. Since women have their anus close to the urethra, and men have an additional appendage to keep their urethra away from the anus, UTIs are more common in women. And women tend to get the bugs of the GI tract causing their UTIs. What isn't so easily connected is that *Klebsiella* is **associated with aspiration pneumonia**. *Klebsiella* pneumonia presents with a necrotic destruction of alveolar spaces, the formation of cavities, and the production of blood-tinged sputum. Combine

the mucoid sputum (like the mucoid colonies) with being blood-tinged, and you get the **currant-jelly sputum** so classically associated with *Klebsiella pneumoniae.* Those who suffer from aspiration pneumonia will be **alcoholics** or those with **oropharyngeal dysphagia** (central nervous lesions). Treatment is with **ceftriaxone** or other penicillin that covers Gram negatives (amoxicillin).

270+. *Serratia* and *Enterobacter* are *Klebsiella*, cause *Klebsiella* diseases, but are urease negative and motile. They are the other lactose fermenters that we've been alluding to.

Proteus

Proteus mirabilis is the last of the common UTI bugs. It is urease positive. Urease cleaves urea. Urease cleaves urea to form NH_4^+ and CO_2, raising the pH of the urine. Increased concentrations of an ion such as ammonium (NH_4^+) predisposes the formation and precipitation of a salt. When salts precipitate out in the urine, we call them kidney stones, nephrolithiasis. Ammonium forms a salt with magnesium and phosphate, to form **magnesium ammonium phosphate** nephrolithiasis (also called struvite kidney stones and staghorn calculi). These are massive kidney stones that form in the pelvis and calyces (more in Renal: Kidney #6: *Kidney Stones*).

Because *Proteus* is **very motile**, it causes a phenomenon called "swarming motility" on regular blood agar (you will see rings on regular agar). It does produce **H_2S**, so will create black pigment on TSI agar. It does not ferment lactose.

Pseudomonas

While there are over 200 species of *Pseudomonas*, only one matters for human disease—*Pseudomonas aeruginosa*, which we refer to here simply as *Pseudomonas*, since it is the only species of medical importance. Wherever water is, *Pseudomonas* can be. It is found in soil, decaying organic matter, vegetation, water, cut flowers, sinks, toilets, floor mops, and respiratory therapy equipment. It is the bane of hospital administrations and is the cause of many nosocomial infections. So virulent is this bacterium that we have designated certain antibiotics "antipseudomonal." *Staph. aureus* and *Pseudomonas* are the only bacteria for which we specifically call out an antibiotic designed to target a single organism.

Structure/physiology. *Pseudomonas* bacteria are **strict aerobes—**they must have oxygen to live. They generate energy through **aerobic respiration**, burning carbohydrates to generate ATP, with oxygen as the final electron acceptor. That means they are **oxidase positive**. *Pseudomonas* produces a **blue-green pigment**, a combination of blue pigment called pyocyanin (cyan = blue) and a green pigment called pyoverdin (verde for green). This makes the colonies identifiable to the naked eye, which greatly accelerates the initial identification. It does not ferment lactose. There is a **fruity** or **grape-like odor** to the colonies as they grow. *Pseudomonas* is **motile**, using **flagella** to move. It is **capsulated** with a mucoid polysaccharide called alginate.

Virulence. *Pseudomonas* has many virulence factors, including adhesins, toxins, enzymatic proteins, and a type 3 secretion system. **Adhesins** come in the way of pili. **Secreted toxins** come in the form of **exotoxin A**, which inactivates EF-2, leading to tissue necrosis. A type 3 secretion system allows the bacteria to deliver exotoxin A directly into the host cell. **Enzymes** are numerous—pigment, elastase, protease, and phospholipase. The blue pigment, pyocyanin, is more than just a color. Pyocyanin is superoxide dismutase—the bacteria generate superoxide and hydrogen peroxide the way human phagocytes do within lysosomes. **Elastases** degrade elastin, resulting in the damage to elastin-containing tissues (like the lungs). **Alkaline protease** contributes to tissue destruction and spread. **Phospholipase C** degrades the membranes of host cells, contributing to further progression of disease.

Diseases. *Pseudomonas* loves water. It causes opportunistic infections of people who contact contaminated water. That leads to pneumonias (respiratory therapy inhalers), burn wounds, folliculitis (hot tubs), otitis externa (swimming pools), and skin infections (diabetics and sneakers). We discuss each briefly, one by one.

Nosocomial pneumonia. *Pseudomonas* is everywhere in a hospital, especially on ventilators. Any patient that has a nosocomial pneumonia (all forms of pneumonia related to being in or having been in a hospital, but whose full name is being withheld until clinicals) is empirically treated for *Pseudomonas*. We cover antipseudomonal β-lactams in Antibacterials #2: *β-Lactam Cell Wall Inhibitors*.

Burn wounds. *Pseudomonas* colonization of a burn wound, followed by localized vascular damage, tissue necrosis, and ultimately bacteremia, is common in patients with severe burns. The moist surface of the burn and inability of neutrophils to penetrate into superficial compromised tissue predispose patients to infections. Proper wound care is essential, though topical antibiotics and ointments are generally insufficient to prevent pseudomonal infections, should they occur. Often, severe burn victims have necrotic tissue washed off in a specialized tub—exposing them and their compromised epidermis to water.

Hot tub folliculitis. *Pseudomonas* loves water and can survive in mildly chlorinated water. Immersion in contaminated water (hot tubs, whirlpools, swimming pools) can result in a diffuse folliculitis. Infection risk increases in those who shave their legs or have acne. *Pseudomonas* can also cause infection of nails of those who frequent nail salons. There isn't anything special about hot tubs, but since the disease has been named hot tub folliculitis and pools (swimming), and is associated with swimmer's ear (below), learn hot tubs and folliculitis in relation to *Pseudomonas*.

Diabetic foot wounds and penetration through sneakers. Sneakers have rubber, which can retain moisture, where *Pseudomonas* can live. Uncommon in life but frequented on examinations is the patient who suffers a puncture wound through a sneaker. Common in life is the diabetic with diabetic neuropathy who has a foot wound. In this sense *Pseudomonas* is the cause of cellulitis (surrounding the wound) but may also cause **osteomyelitis** if the infection penetrates to the bone. Until cultures say otherwise, empiric treatment for *Pseudomonas* is started.

UTIs. *Pseudomonas* UTIs are associated with long-term **indwelling urinary catheters** (though they are not common in the general population, as poop bugs go up the urethra but *Pseudomonas* does not). These patients both provide plastic on which bacteria can grow, and suffer from recurrent UTIs, whose treatment with repeated antibiotics advances the natural selection towards more-resistant strains of bacteria, such as *Pseudomonas*. *Pseudomonas* makes a biofilm, which easily binds to nonorganic matter such as plastic.

Otitis externa. Ear infections that do NOT involve the middle ear (inside) and involve only the outer ear, can be caused by *Pseudomonas*. Commonly referred to as **swimmer's ear**, the pinna is red, inflamed, and painful. This is treated with topical antibiotics (ciprofloxacin drops) and drying agents. Malignant external otitis is a virulent form of the disease that involves erosion and invasion of surrounding tissue, associated with diabetes and the elderly.

Corneal ulcers. Using water only for contact lens solution is a bad idea. *Pseudomonas* grows in the water, the contact goes onto the eye, and bam, *Pseudomonas* grows in the cornea.

Treatment. Antipseudomonal antibacterials include pip/tazo, cefepime, carbapenems, and aztreonam—all β-lactam derived. *Pseudomonas* pneumonia should be double covered—any one agent plus ciprofloxacin. Ciprofloxacin should never be used on its own to treat *Pseudomonas*, except in the case of otitis externa, where ciprofloxacin drops are used. Tobramycin inhalation treatment is used to treat cystic fibrosis pneumonia.

Legionella

Acquiring its name from the outbreak that claimed many lives amongst the American Legion members at a convention in Philadelphia, this sneaky Gram negative had been avoiding detection and attention for all of human existence. It is a ubiquitous aquatic saprophyte—it's everywhere and loves environmental water sources. It evaded detection because it does not stain with conventional dyes and does not grow on any media but the medium designed specifically for it. Whereas *Pseudomonas* is found in water, *Legionella* is found in water tanks.

Structure/physiology. *Legionella* bacteria are **slender, pleomorphic** Gram-negative rods. It is hard to grow *Legionella*. It requires oxygen (obligative aerobic) and special nutrients (needs to be fed cysteine and iron). It is primarily an **intracellular organism** reliant on the host cell for energy. In the environment, it grows and replicates within amoeba. In humans, it grows and replicates within alveolar macrophages and alveolar epithelial cells. The organism stains poorly because it is primarily an intracellular organism, though it can be seen with a silver stain. To grow, it requires the use of **charcoal yeast extract agar** buffered with cysteine and iron.

Epidemiology. *Legionella* is spread to humans via aerosol transmission from environmental water sources. Person-to-person transmission is rare. Cases are reported in the summer months, when people are more likely to expose themselves to aerosolized water sources—air conditioners, misters, sprinklers. Those at most risk have weakened immunity of the lungs—smokers, the elderly, and the immunocompromised.

Diseases. Most of the infections by *Legionella* are thought to be asymptomatic. Symptomatic infections primarily affect the **lungs**, and present in one of two forms—an influenza-like illness referred to as Pontiac fever (named after the town of Pontiac, Michigan, where people in the public health department fell ill with the condition) and a severe form of pneumonia known as **Legionnaires' disease**.

Pontiac fever is rarely diagnosed. The patient has a self-resolving, nonsevere rise in their temperature, vague symptoms of being infected, and no consolidation on chest X-ray, and then spontaneously get better. There is no reason to look for *Legionella* antigen, so no one does. Without confirmation of the causative organism, making the diagnosis is impossible. Most patients don't even seek medical attention.

Legionnaires' disease is a severe pneumonia plus. The plus is diarrhea, altered mentation that is inappropriate for the level of hemodynamic compromise, and a profound hyponatremia amongst normal labs. These symptoms are not specific enough to make the diagnosis in life, but are on the test. Classic pneumonia has fever, cough, and consolidation on chest X-ray, but no GI symptoms, CNS symptoms, or hyponatremia. Finding excess information about the GI and CNS may point you towards Legionnaires' disease. Diagnosis is made with a **urinary antigen**. *Legionella* causes pneumonia, so you look in the urine for antigen. That disconnect makes *Legionella* testing a favorite of board examinations.

Treatment. Macrolides (**azithromycin**) or fluoroquinolones can be used to treat it. When empirically treating ambulatory pneumonia or bronchitis, the common choices are azithromycin or moxifloxacin. Routine treatment of routine pulmonary infections often covers *Legionella* even without the diagnosis confirmed.

BUG	NOTES
E. coli	Lactose fermenter, MacConkey pink, unique metallic green sheen on EMB K1 capsular antigen (meningitis), P-pilus adhesin (UTIs) Heat-stable and heat-labile enterotoxins (watery diarrhea), Shiga toxin (bloody diarrhea)
Klebsiella	Lactose fermenter, MacConkey pink, EMB purple Urease positive, causes UTIs and can cause struvite stones Aspiration pneumonia in alcoholics; currant-jelly sputum
Proteus mirabilis	Very motile (swarming motility), H_2S turns TSI black Urease positive, causes struvite stones (staghorn, magnesium ammonium phosphate) Alkalinizes urine, causes UTIs
Pseudomonas	Oxidase positive, strict aerobe, grows blue-green colonies, colonies smell like grapes Motile, have flagella, have a capsule Adhesins—pili, capsule, flagella Toxins—exotoxin A (EF-2 inhibition, death of host cell) and type 3 secretion system Enzymes—elastase kills lungs, protease and phospholipase dissolve human cells Nosocomial pneumonia—pip/tazo and ciprofloxacin Otitis externa, swimmer's ear—ciprofloxacin eye drops Diabetic foot wounds, sneaker penetration injury, osteo—pip/tazo
Legionella	Small, intracellular, so do not stain well, and must be grown on charcoal yeast extract Legionnaires' disease—regular pneumonia PLUS gastro and CNS symptoms, dx by urine Ag Pontiac fever, mild flu-like illness Azithromycin for Legionnaires', symptomatic for Pontiac fever

Table 8.3: Bacterial Summary